Role of Glutamate and Glutamate Receptors in Memory Function and Alzheimer's Disease[a]

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Several investigations stress the involvement of glutamate and its receptors in learning and memory. The hippocampal region attracted early attention for being involved in such processes in man.[1,2] Later, long-term potentiation (LTP) was first ascribed to the hippocampus and several studies have developed the concept as a synaptic model for memory.[3,4] LTP is blocked by *N*-methyl-D-aspartate (NMDA) receptor antagonists and is localized to several different glutamergic pathways.[5] LTP is, however, a widespread phenomenon and can be produced throughout the limbic forebrain including the entorhinal area, but marked differences exist between different pathways.[6] Further, animal studies involving selective hippocampal lesion have not been able to confirm a global mnemonic role of the hippocampus.[7] According to the literature on the rat, the hippocampus appears to be more involved in working memory than in reference memory.[8,9]

Human investigations on memory function focused on the neurochemical and histological changes in Alzheimer's disease. Alzheimer's disease is a form of dementia characterized histologically by the presence of neurofibrillary tangles and senile plaque and a loss of pyramidal cells in the brain. The severity of the dementia is related to the number of neurofibrillary tangles and less to the senile plaques. Previously, the cholinergic dysfunction in Alzheimer's disease had been the dominating concept,[10] but in the last five years several reviews have pointed out a possible central role of glutamergic neurons in the development of the pathology.[11–14] The ventromedial temporal lobe including the entorhinal cortex, the hippocampus, and the amygdala are the most markedly atrophied regions in Alzheimer's disease. The association areas of the parietotemporal and prefrontal cortex are involved to an intermediate degree.[15,16]

It now seems accepted that the first cells to be affected with neurofibrillary tangles are located in the transentorhinal cortex, followed by the perirhinal cortex, the entorhinal cortex, and the pyramidal cells in CA1 and subiculum. This hierarchy of pathology occurs both in normal aging and more predominantly in Alzheimer's disease.[17,18] The affected areas in the brain seemed to be closely linked with the entorhinal cortex. An area separated by none or one synapse is more affected than an

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area separated from the entorhinal cortex by several synapses.[19] The entorhinal cortex is linked with strong projections from the association and limbic cortices.[20]

In view of the emphasis placed on entorhinal and perirhinal cortices, we investigated some glutamergic parameters after surgical transections involving these regions. Further, the effects of rearing animals under different conditions have also been investigated both neurochemically and in terms of cognitive functions. Most importantly, however, we investigated the effects of surgical lesions on learning and particularly on retention of memory. Finally, we showed how glutamatergic agonists can ameliorate the effect of lesion on learning and retention of memory and discussed their possible therapeutic potential in treatment of Alzheimer's disease.

METHODS

Rats were examined in a visual discrimination test in which the task was to differentiate one aluminium cylinder, either grey or black, from two other aluminium cylinders, which were in the other shade.[21] Testing was carried out in a Plexiglas cage (56 × 34 × 20 cm). A Plexiglas wall with an opening (10 × 10 cm) in the middle divided the apparatus into two equal compartments: the start compartment and the goal compartment. In the goal compartment there were three interchangeable aluminium cylinders with a round well in the top. The cylinders were placed with equal distance between them along the wall opposite to the partition wall. Half of the animals were trained with the black cylinder as positive and the other half with the grey cylinder as positive. The position of the positive cylinder (left, middle, right) was changed in a prearranged order and by a different set of randomized positions on days 2 and 3 and on retention. The positive cylinder was the same during all the testing for each animal.

During acquisition and retention testing the rats were deprived of water for 23½ hours a day. On the first day, each rat was allowed to explore the empty test apparatus for 15 min. On the second day, the rats were trained to run from the start to the goal compartment and allowed to explore the cylinders until they hit the positive one with water. The rats were given 10 trials and the intertrial interval was 20 s. On the third day, the animals were tested until five correct responses in succession were obtained. Both the number of errors and the number of trials until the learning criterion had been reached were noted. In order to drink or to investigate whether the well contained water, the rat had to stand on its hind legs with at least one forepaw on top of the cylinder. Error was scored when the animal mounted a negative cylinder to drink and not when they only investigated the cylinders apart from the well. The animals were operated on day 4 as described below. Intraperitoneal injections of glutamatergic agonists were given immediately after the operation and at two and ten days after surgery. The animals were tested for retention 12 days after surgery, that is, on day 16.

The animals were first operated on for the learning paradigm. Eight days later they were trained with the same procedure as described above.

Surgery

The rats were anesthetized intraperitoneally with diazepam (10 mg/kg) and fentanyl fluanisone (2 mg/kg) and placed in a stereotaxic head holder. The lesions were made mechanically by means of the sharp edge of cannula (diameter 0.5 mm) as described.[21]

Neurochemical Assays

High-affinity D-aspartate uptake was taken as a marker for glutamergic nerve terminals and assayed as described by Lund Karlsen and Fonnum.[22] Choline acetyltransferase and glutamate decarboxylase were used as markers for cholinergic and GABAergic nerve terminals, respectively. Choline acetyltransferase and glutamate decarboxylase were assayed by previously described methods.[23,24] Tissue samples were taken from the temporal cortex (TC), the lateral entorhinal cortex (LEC) or the frontal cortex.

RESULTS AND DISCUSSION

Neurochemical Studies of Entorhinal and Temporal Cortices

The importance of the entorhinal and temporal cortices in cognitive and mnemonic processes prompted us to investigate some neurochemical parameters in these regions. We found a 20% higher level of high-affinity D-aspartate uptake in a homogenate from the left LEC than from the right side in normal rats ($p < 0.05$). Such a lateralization was not found in the TC or for the other parameters such as choline acetyltransferase and glutamate decarboxylase.[25] This lateralization is also consistent with the effect of unilateral lesion on retention of memory (TABLE 1).

TABLE 1. Changes in Retention of a Visual Discrimination Task following Transections of Temporoentorhinal Connections

Lesions	Prior Learning Trials (*n*)	Retention (12 days)	
		Trials (*n*)	Errors (*n*)
No lesion	18	6.5	1.4
Bilateral TC/LEC	18	22.7[a]	8.6[a]
Left TC/LEC	16	12[a]	3[a]
Right TC/LEC	17	5	0
Bilateral medial perforant path	18	6.3	1.1
Bilateral lateral perforant path	19	7.4	1.3
Bilateral dorsal hippocampus	18	6.9	1.4

Abbreviations: TC, temporal cortex; LEC, lateral entorhinal cortex.
[a] $p < 0.05$.

Further support for the lateralization of glutamergic activity in LEC is the consistent enlargement of the lesions in the left LEC after systemic administration of glutamergic agonists.[26] When the TC/LEC connections were transected at the level of the perirhinal cortex, a 40% reduction in high-affinity D-aspartate uptake in both denervated cortices, demonstrating a reciprocal glutamergic connection ($p < 0.01$). No corresponding effects on glutamate decarboxylase or choline acetyltransferase were found. The remaining glutamergic activities in LEC and TC indicate other glutamergic inputs, for example, from associative cortical connections.[27]

It is well known than environmental factors can modify the development of the central nervous system. Rosenzweig and co-workers showed that rats reared in an enriched environment had increased brain weight, higher acetylcholinesterase activity, and an increased number of dendritic spines compared to rats housed in an

isolated environment.[28] We focused on the effect of different rearing conditions on the glutamergic and cholinergic systems in entorhinal and temporal cortices[29] (TABLE 2). Three groups of rats (25 days old) were reared under isolated, social or enriched conditions for two months. The isolated rats were reared in a Plexiglas cage alone. The social rats were reared in groups of five in a large Plexiglas cage. The enriched group was reared in groups of five in a similar large cage containing three objects that were changed three times a week.

A significant difference in D-aspartate uptake was present in LEC, in correlation with the more enriched rearing conditions (TABLE 2). The enriched group showed a significant increase compared to the isolated group ($p < 0.01$). In the enriched group there was slightly higher uptake activity in LEC than in both temporal and frontal cortices. No differences in choline acetyltransferase were found between the different groups or between the three cortical areas. The enriched group had a slightly lower body weight than the other two groups.[29]

TABLE 2. Biochemical Parameters and Cognitive Function in Lateral Entorhinal Cortex of Rats Reared under Different Conditions

A. Biochemical Parameters			Isolated (10)[a]	Social (10)	Enriched (10)
HA DAsp uptake			1261	1436	1579
ChAT			527	528	519
	Day 1	Acquisition (Day 2)		Retention (Day 14)	
B. Cognitive Function	Errors[b]	Errors[b]	Trials	Errors	Trial[b]
Isolated (7)	4 (2–5)	1 (0–2)	17 (15–21)	1 (0–2)	8 (5–11)
Enriched (8)	2 (1–3)	0 (0–1)	15 (15–16)	0 (0)	5 (5–7)

[a]Number of samples indicated in parentheses.
[b]$p < 0.05$.

The level of D-aspartate uptake correlated significantly with both the acquisition and the retention of the brightness discrimination task (TABLE 2). These findings need not mean that uptake activity or glutamergic terminals in LEC are casually related to behavior. Together with other findings in this paper, however, they strengthen the case for an involvement of LEC in learning and memory.

Studies on Learning and Retention of Memory after Brain Lesions

We studied the acquisition and retention of memory in a series of surgical lesions including entorhinal cortex, temporal cortex, perirhinal cortex, and hippocampus.[21,30] Transection of the white matter in the rhinal sulcus disrupts the reciprocal connections between TC, perirhinal cortex, and LEC. Bilateral transections resulted in a dramatic impairment in retention of the visual discrimination task (TABLE 1). When unilateral transection was carried out involving the left side, the one with the highest D-aspartate uptake activity, a marked reduction also occurred in retention of the task. Transection involving the right-hand side showed no effect or even a slight improvement in the retention of this task.

A corresponding retention deficit could not be observed with a bilateral transection of the medial or lateral perforant path or by a bilateral removal of two-thirds of

the hippocampus (TABLE 1). The lesions involving the perforant path, however, affect the animal's exploratory behavior and reaction to novelty.[31]

Role of Glutamergic Receptors in Cognitive Function and Alzheimer's Disease

The glutamergic receptors are separated into NMDA receptors, AMPA/kainate receptors, and metabotrophic receptors.[32] The NMDA receptor consists of several subunits called NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C, and NMDAR2D. Heteromeric assemblies of these subunits give receptors that react very differently with glycine.[33] The AMPA/kainate receptor consists of several subunits, some with high affinity for AMPA and some with high affinity for kainate.[34] So far, six or seven metabotrophic receptors have been identified.[35]

Several examples in the literature show that NMDA receptor antagonists will block learning. The most prominent are probably the works of Morris and colleagues[36,37] who showed that intraventricular infusion of the antagonist DLAP-5 caused an impairment of spatial learning, but not the retention of previously acquired spatial information. It also blocked LTP *in vivo* without blocking normal synaptic transmission. Ingram *et al.*[38] showed a dose-dependent impairment of maze performance after giving dizocilpine (MK801). Watanabe *et al.*[39] showed that intraventricular injection of 7-chlorokynurenic acid, a selective antagonist at the glycine site of the NMDA receptor, inhibited LTP and impaired spatial learning in rats.

Several studies exist on changes in the sodium-independent glutamate binding, that is, receptor binding, in brains of patients with Alzheimer's disease. Earlier studies, using less specific assays, showed a dramatic fall in NMDA receptors in the hippocampus and parahippocampal region.[40] The issue was reexamined with 10 Alzheimer brains, nine controls, and six demented brains. Overall, there was a 50% loss of NMDA receptors in the pyramidal cell layer of CA1 and a 40% loss in CA3 compared to control and demented brains.[41] The decrease in NMDA receptor binding was confirmed in an investigation of a number of very old, nondemented or demented women and younger control brains[42] (27–64 years). A reduction in NMDA and kainic acid receptor binding was found in very old, nondemented women compared to controls, and a further significant reduction was found in Alzheimer brains. There was also some correlation with mental status, the number of neurofibrillary tangles, and the loss of receptor binding in CA1 of hippocampus. Jansen *et al.*[43] found a decrease of 50% in NMDA receptor binding, binding to the ion channel and to the glycine site in the CA1 region of hippocampus. Other studies have not been able to confirm a loss of NMDA receptors in hippocampus in Alzheimer's disease.[44–46]

Procter *et al.*[47] studied ^{3}H[MK801] binding to the ion channel of the NMDA receptor and reported reduced glycine stimulation in samples from frontal, parietal, and temporal cerebral cortex of Alzheimer's disease brains. This finding was later challenged.[43,48] Glycine binding can easily escape detection in membranes that are not well washed or contain an endogenous agonist. In the three cases above, a reduction of 30–50% occurred in the fully stimulated NMDA receptor binding. There was no significant loss in the parietal and temporal cortices.[47]

In general, there is a reduction in NMDA receptor binding in the hippocampus and parahippocampal regions. Little evidence supports the view that the properties of the receptor have been altered. One should keep in mind that there are several forms of the NMDA receptor, which will differ slightly in their properties. Although

this has not yet been demonstrated, it may be that some forms of NMDA receptors are more resistant to loss than others during Alzheimer's disease.

Both Jansen *et al.*[43] and Dewar *et al.*,[49] but not Penney *et al.*,[41] found a loss of AMPA binding in CA1 and the subicular regions of the hippocampal and the parahippocampal regions. It is in this context interesting to note that aniracetam, a so-called nootropic drug, improved synaptic neurotransmission mediated by AMPA receptors.[50] Because the AMPA receptor is often colocalized with the NMDA receptor, a loss of both receptors is not surprising.

Dewar *et al.*[49] found a reduction in glutamate metabotrophic receptor binding in CA1, and the loss was larger than could be accounted for by the loss of neurons. The metabotrophic receptors may play a role in the release mechanism of neurotransmitters.

The memory dysfunction in rats with TC/LEC lesions could be effectively ameliorated by intraperitoneal injection of NMDA or AMPA/kainate receptor agonists (TABLE 3). The agonists were given one day after acquisition of the task and

TABLE 3. Effect of Glutamergic Agonist on Retention of Visual Discrimination Task in TC-LEC Transected Rats

	Prior Learning		Retention (12 days)	
	Dose (mg/kg)	Trials (*n*)	Trials (*n*)	Errors
Untransected + saline		18.8	6.8[a]	0.5
Transected +				
Saline		17.3	22.5	9.5
NMDA	50	18.0	7.0[a]	0.8
Glycine	750	17.5	5.7[a]	0.3
AMPA	2.5	19.3	6.8[a]	0.7
Kainate	5	17.6	14.7	4.2
Adrenaline	0.1	16.8	19.7	6.0
Bretylium + glycine	5 + 750	17.6	6.0	0.5
HA966 + glycine	30 + 750	17.8	18.5	8.2

TC, temporal cortex; LEC, lateral entorhinal cortex.
[a] $p < 0.01$.

thus immediately after the surgical transection. The chemical compounds were also given two days after and sometimes ten days after the lesion. They were also given in high enough doses to pass through the blood-brain barrier. The glycine concentration used was high enough to cause temporary paralysis of the hind legs for a few minutes, and kainic acid produced "wet dog shakes." Both NMDA and glycine restored the memory retention.[51] When glycine was used in half the concentration (375 mg/kg), it had a weaker effect.[26] When HA-966, an antagonist of the glycine site in the NMDA receptor, was given 30 min before glycine, no restoration of memory function was found (Myhrer, unpublished data).

The agonists did not act by way of a peripheral adrenergic mechanism because blockade of adrenergic release with bretylium did not reduce the positive effect of glycine.[51] AMPA had a positive effect, whereas kainic acid, which had an additional toxic effect, was less effective. The results showed that both NMDA receptor agonists affecting the glycine site of the NMDA receptor and AMPA/kainate receptor agonists restored the memory function after surgical lesion of TC/LEC. Full improvement was also seen in proactive memory when glycine was given prior to training.[26]

While this work has been in progress, several other reports have been published that support a positive effect of glutamate agonists in learning and memory in animal experiments. In mice that were trained on a shock avoidance learning paradigm, it was shown that intraventricular injection of L-glutamate, L-aspartate, (±)β-*p*-chlorophenylglutamate, kainate, and quisqualate improved retention after one week in a dose-dependent manner, whereas D-glutamate did not.[52] Similarly, milacemide, a glycine prodrug, enhanced performance in a learning task in normal and amnestic rodents.[53]

Experiments with Cycloserine, a Partial NMDA Agonist

D-Cycloserine is a potent partial agonist which acts on the glycine site of the NMDA receptor. It possesses 60% of the maximal response of glycine and passes easily through the blood-brain barrier.[54] Cycloserine stimulates MK-801 binding to the NMDA receptor equally efficiently in membranes from the inferior parietal cortex of both control and Alzheimer brain.[55] Cycloserine has been used as an antibiotic against tuberculosis and has been given to humans in rather high concentration (0.5–1.0 g). When cycloserine was given in a single dose to TC/LEC transected rats, it effectively restored memory provided it was given within a few days after the operation or just prior to the retrieval. It is suggested that the action profile of cycloserine may reflect effects of both functional (LTP) and pharmacological mechanisms.

Monahan *et al.*[54] was the first to show that the partial agonist cycloserine (3 mg/kg) could improve learning and memory in a one-trial passive avoidance test and in reversal of T-maze learning. Sirvio *et al.*[56] and Fishkin *et al.*[57] extended Monahan's findings by using rats pretreated with scopolamine (1 mg/kg) in a water-maze test and found improvement in performance with varying doses of cycloserine. Flood *et al.*[58] obtained improvement in performance of a footshock test with cycloserine for both young and senescent rats treated with different doses of scopolamine. Schuster and Schmidt[59] obtained positive results with cycloserine (12 mg/kg) given to rats with hippocampal lesions 30 min before testing of working memory in an allocentric reversal test.

In contrast, Rupniak *et al.*[60] examined the effect of cycloserine on primates treated with scopolamine and did not find any improvement in a visual-spatial memory test. The concentration of cycloserine used ranged from 3 to 14 mg/kg. When we compare this dose to that given to humans (see next section), this may be too high a dose. An overview of studies involving cycloserine in cognitive function is presented in TABLE 4.

Experiments of Cycloserine in Humans

Twenty-four healthy, young male and 24 healthy, elderly male and female (age 63–75) volunteers participated in two identical studies using a double-blind placebo-controlled Latin square design.[61] The participants received scopolamine 0.5 mg (young) and 0.2 mg (old) and later 0, 5, 15 or 50 mg cycloserine. For young subjects, 15 mg D-cycloserine antagonized the scopolamine-induced decrement in three memory tasks. Less or no significant effects were seen with 5 or 50 mg doses. In older subjects, 5 mg cycloserine significantly reduced the decrement in a word recognition test. A similar but smaller effect was seen with 15 mg, whereas 50 mg was inefficient.

TABLE 4. Experiments Involving Cycloserine in Cognitive Function

Authors	Species	Status		Cycloserine (mg/kg)	Results
Monahan *et al.*[54]	Rat	Normal	Passive avoidance Reversal of T-maze	3	+
Sirvio *et al.*[56]	Rat	Scopolamine	Water maze		+
Fishkin *et al.*[57]	Rat	Scopolamine	Water maze	3, 10, 30	+
Flood *et al.*[58]	Rat	Senescent rats	Foot shock	20	+
Schuster & Schmidt[59]	Rat	Hippocampal lesion	8-Arm maze	12	+
Rupniak *et al.*[60]	Primates	Scopolamine	Visual-spatial memory	3–14	−
Jones *et al.*[61]	Human	Scopolamine	Several tests	5, 15 mg[a]	+

[a]Dose per individual.

We (Wangen *et al.*, unpublished data) studied the effects of D-cycloserine (0, 5, and 15 mg) on six persons with Alzheimer's disease (TABLE 5). The patients were not on medication, and the symptoms ranged from those of a recently acquired illness to severe symptoms of Alzheimer's disease. The patients were nursed at home by their families. The patients received in three consecutive weeks placebo tablets, 5 mg tablets or 15 mg tablets, in a prearranged order. The studies were carried out double blind. The patients were tested before the study started to obtain the baseline level and during the last day on receiving each dose. The results show that five of six patients responded better with both doses of D-cycloserine than with placebo in the trial-making test. In the object-learning test three were better with the low dose, one was better with the high dose and the remaining two were equal to the placebo. In one instance the family reported a significant improvement in behavior, and also in another case there was also some improvement during cycloserine administration.

TABLE 5. Clinical Testing of Low or High Dose of D-Cycloserine in Patients with Alzheimer's Disease[a]

	Object Learning Test[b]				Trial Making Test[c]			
			Cycloserine				Cycloserine	
Patient	Base	Placebo	Low	High	Base	Placebo	Low	High
AA	36	33	38	32	50	42	27	32
BB	22	21	34	19	65	66	51	46
CC	25	27	28	23	119	58	83	64
DD	14	23	23	17	510	241	127	198
EE	19	23	20	23	144	217	139	184
FF	16	18	18	21	66	61	58	49

[a]Low dose of D-cycloserine = 5 mg; high dose = 15 mg.
[b]Expressed in points.
[c]Expressed in seconds.

CONCLUDING REMARKS

In this paper we focused on the glutamatergic connections between entorhinal and temporal cortices and their importance for learning and, more significantly, for

retention of memory. The glutamergic transmission in the lateral entorhinal cortex is subjected to a slight degree of lateralization and also is affected by the conditions of rearing the animals. In both cases increased glutamergic activity correspond to an improved ability of learning and retention of memory. Administration of glutamergic agonists, both of the NMDA and AMPA types, restores the mnemonic function following a transection of the connection between entorhinal and temporal cortex. Several studies show cycloserine, a partial NMDA agonist, is effective in improving mnemonic processes in man and animals with brain lesions or manipulated with scopolamine, a muscarinic antagonist. The study shows that cycloserine or similar compounds may have a positive effect on patients with Alzheimer's disease and provides a basis for investigating further such a therapy.

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